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Control of inflorescence architecture by AP01 and AP02 genes in rice

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博 士 論 文

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***APO1* and *APO2* genes in rice**

(イネ花序構造における *APO1* と *APO2* 遺伝子の制御機構)

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植物発生分野

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Background

A fascinating feature of plant growth and development is that plants initiate organs continually throughout their lifetime. This unique feature relies on activity of meristems that contain pluripotent stem cells and allow elaboration of the shoot, root, and vascular systems. During rice growth, growth phase changes from vegetative phase to reproductive phase. Accompanying the change, the shoot meristem changes its fate from shoot apical meristem (SAM) to inflorescence meristem (IM), which produces primary branch meristems (PBM), secondary branch meristems (SBM) continuously, and finally spikelet meristems (SM). A large number of genes are involved in the control of meristem phase change and subsequent inflorescence development, *ABBERANT PANICLE ORGANIZATION 1* (*APO1*), the rice ortholog of Arabidopsis *UFO* encoding an F-box protein, and *APO2*/*RFL*, the rice ortholog of Arabidopsis *LEAFY*(*LFY*) encoding a plant-specific transcription, play key roles in the control of the rice inflorescence architecture. Because the downstream genes of *APO1* and *APO2* are still unclear, I aim to understand how *APO1* and *APO2* regulate inflorescence development in rice.

Results

1. Phenotype of *apo1-1*(N) and *apo2-1*(N) mutants.

The phenotypes of loss-of-function mutants of *APO1* and *APO2* in Nipponbare background were observed under the natural growth condition. In the entire life cycle, both *apo1-1*(N) and *apo2-1*(N) mutants showed increased leaf number, more tiller number, shorter plant height and thinner internodes than wild type as previously reported. Inflorescence of rice is called as a panicle. For the panicle architecture, both *apo1-1*(N) *and apo2-1*(N) mutants caused the precocious conversion to the floral meristem therefore generated smaller panicles with less spikelets. In addition to the known phenotypes of *apo1* and *apo2* loss-of-function mutants, I found novel phenotypes. In both *apo1-1*(N) and *apo2-1*(N) mutants, an overgrowth bract formed at the bottom of each panicle was observed. Mutants of *UFO* and *LFY* in Arabidopsis also produced an additional bract structure around flower, suggesting that the repression of bract development controlled by *LFY*/*UFO* is conserved in Arabidopsis and rice.

I also grew plants under completely controlled environments to compare photoperiod sensitivity. Plants were transferred to short day condition at 8th leaf stage and the time-course of the change of meristem morphology was histologically examined. I found that enlargement of SAM, suggesting the transition to IM phase, occurred at 10 days after the transfer to SD condition in wild type and this was not affected in *apo1-1*(N) and *apo2-1*(N) mutants. The meristem size was comparable between wild type and *apo1-1*(N) and *apo2-1*(N) mutants until transition, whereas the meristem size after the transition was much smaller in the mutants than that in wild type.

This part of work demonstrated that *APO1* and *APO2* are not involved in photoperiod sensing. On the other hand, they positively regulate proliferation of the meristem cells at the transition.

2. Analysis of genes working downstream of *APO* and *APO2* in the control of reproductive transition.

The transition from a vegetative to a reproductive state, also called reproductive transition, is the first step of inflorescence development. I analyzed changes of gene expression profiles in the meristem from the start of SD treatment to reproductive transition by laser microdissection (LMD) RNA sequencing (RNA-seq). The clustering during reproductive transition in wild type showed various expression trends and predicted clusters could represent the enrichment of each sampling time point (Fig 1). It was shown that the biggest change of gene expression was observed at 10 days when reproductive transition occurred. The highest number of genes including *APO2* were classified as Cluster 6, in which gene expression was specifically upregulated at 10 DAY. By combining with the DEGs detected from comparison among four sampling stages, several presentative genes were detected, including *OsMADS51*/*65*, *OsPCL1*, *OsSPL14* and *NL1*/*OsGATA15*.

I also examined the differences in the transcriptomes in the IM of wild type, *apo1-1*(N) and *apo2-1*(N) mutants.

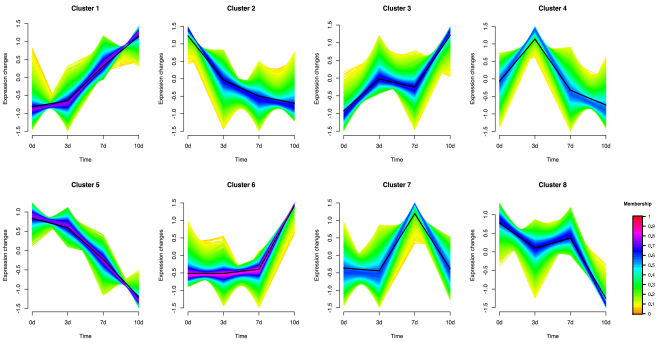

Fig 1. Gene expression patterns heading for reproductive stage.

GO analysis revealed that upregulated DEGs in *apo1-1*(N) *and apo2-1*(N) mutants were enriched in the response to external stimulus while downregulated genes were closely correlated to flower development, reproductive system development. Cell cycles genes, phytohormone (GA, auxin and cytokinin) pathway related genes were detected from DEGs of *apo1-1*(N) and *apo2-1*(N) mutants. In addition, a large number of panicle development associated genes were detected only in downregulated DEGs of *apo1-1*(N) and *apo2-1*(N) mutants.

3. Direct downstream genes of APO2 transcription factor during branch determinacy transition.

Since APO2 is a transcription factor, APO2 direct binds to *cis* elements to regulate transcription of target genes. However, there is still no clear information about the direct target genes of APO2, especially during panicle development. To explain divert effects of *apo1-1*(N) *and apo2-1*(N) mutants in panicle structure, chromatin immunoprecipitation sequencing (ChIP-seq) analysis was performed within 0-2 mm panicle stage. Sequencing data predicted total 3184 direct downstream genes and three significant binding motifs. Among all target genes, more than 12% belonged to various transcription factor families, such as AP2-EREBP family, WRKY family and MADS family, which suggests that APO2 may control several pathways by regulating distinct downstream genes. Go ontogeny (GO) analysis showed that reproductive system development, cell growth and generation of metabolites and energy were main functions of target genes of APO2. Among 3184 target genes, *NL1*, *OsRA2*, *OsSPL7*, *OsSPL14* and *OsSPL17* were shared with the gene set of enriched genes in 10 DAY (Cluster6) and the down-regulated DEGs in *apo1-1*(N) *and apo2-1*(N) mutants. It is suggested that these genes are positively and directly regulated by APO2 from the reproductive transition to branch transition stage. To verify the binding between APO2 and target genes, *in situ* hybridization and ChIP-qPCR were utilized and results confirmed the direct regulation.

Conclusion

In this study, direct and indirect downstream genes of *APO1* and *APO2* were identified (Fig 2). Direct target genes include number of genes that were already studied as key genes in the control of inflorescence development of rice, such as *OsSPL14* and *NL1*, suggesting that APO2 is a main regulator of rice inflorescence development. I also found that the reproductive transition and branch determinacy transition, two continuous steps of rice panicle development are regulated by APO2. Further research is needed to verify the genetic regulation of direct downstream genes. In particular, it is fascinating to reveal how the progressive changes of distinct meristem phases, from SAM to IM and BM to FM are regulated by APO2.

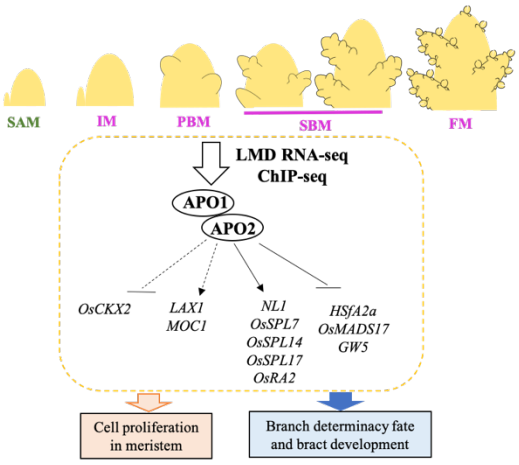

Fig2. Model for regulation of *APO1* and *APO2* during reproductive phase in rice.